

AMENDMENTS TO THE CLAIMS:

The listing of claims below will replace all prior versions, and listings, of claims in the application. These amendments introduce no new matter and support for the amendment is replete throughout the specification and claims as originally filed. These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter, or agreement with any objection or rejection of record.

Listing of Claims:

1 (Currently Amended). A method for synthesis of a glycoprotein, the method comprising:

incorporating, in a translation system, [into a protein] an unnatural amino acid that comprises a first reactive group into a protein; and,

contacting the protein with a saccharide moiety that comprises a second reactive group, wherein the first reactive group reacts with the second reactive group to attach the saccharide moiety to the unnatural amino acid, thereby producing the glycoprotein.

2 (Original). The method of claim 1, wherein the first reactive group is an electrophilic moiety and the second reactive group is a nucleophilic moiety.

3 (Currently Amended). The method of claim 2, wherein the electrophilic moiety is a ketone or aldehyde moiety.

4 (Currently Amended). The method of claim 2, wherein the nucleophilic moiety is selected from the group consisting of: $\text{—NR}^1\text{—NH}_2$ (hydrazide), $\text{—NR}^1(\text{C=O})\text{NR}^2\text{NH}_2$ (semicarbazide), $\text{—NR}^1(\text{C=S})\text{NR}^2\text{NH}_2$ (thiosemicarbazide), $\text{—(C=O)NR}^1\text{NH}_2$ (carbonylhydrazide), $\text{—(C=S)NR}^1\text{NH}_2$ (thiocarbonylhydrazide), $\text{—(SO}_2\text{)NR}^1\text{NH}_2$ (sulfonylhydrazide), $\text{—NR}^1\text{NR}^2(\text{C=O})\text{NR}^3\text{NH}_2$ (carbazide), $\text{—NR}^1\text{NR}^2(\text{C=S})\text{NR}^3\text{NH}_2$ (thiocarbazide), and —O—NH_2 (hydroxylamine), where each R^1 , R^2 , and R^3 is independently H, or an alkyl having 1-6 carbons.

5 (Currently Amended). The method of claim 4, wherein the nucleophilic moiety is selected from the group consisting of: hydrazide, hydroxylamine, semicarbazide, and carbohydrazide.

6 (Original). The method of claim 2, wherein the reaction product comprises an oxime, an amide, a hydrazone, a carbohydrazone, a thiocarbohydrazone, a sulfonylhydrazone, a semicarbazone, or a thiosemicarbazone.

7 (Original). The method of claim 6, wherein the reaction product comprises a reduced hydrazone.

8 (Original). The method of claim 1, wherein the first reactive group is a nucleophilic moiety and the second reactive group is an electrophilic moiety.

9 (Currently Amended). The method of claim 8, wherein the electrophilic moiety is a ketone or aldehyde moiety.

10 (Original). The method of claim 1, wherein the saccharide moiety comprises two or more carbohydrate moieties.

11 (Currently Amended). The method of claim 1, further comprising: c) contacting the saccharide moiety with a glycosyltransferase, and a sugar donor moiety, [and other reactants required for glycosyltransferase activity] for a sufficient time [and under appropriate conditions] to transfer a sugar from the sugar donor moiety to the saccharide moiety.

12 (Original). The method of claim 11, wherein the glycosyltransferase is selected from the group consisting of: a galactosyltransferase, a fucosyltransferase, a glucosyltransferase, an N-acetylgalactosaminyltransferase, an N-acetylglucosaminyltransferase, a glucuronyltransferase, a sialyltransferase, a mannosyltransferase, a glucuronic acid transferase, a galacturonic acid transferase, and an oligosaccharyltransferase.

13 (Original). The method of claim 11, wherein the method further comprises contacting a product of step (c) with at least a second glycosyltransferase and a second sugar donor moiety.

14 (Original). The method of claim 11, wherein the saccharide moiety comprises a terminal GlcNAc, the sugar donor moiety is UDP-Gal and the glycosyltransferase is a β -1, 4- galactosyltransferase.

15 (Original). The method of claim 11, wherein the saccharide moiety comprises a terminal GlcNAc, the sugar donor moiety is UDP-GlcNAc and the glycosyltransferase is a β 1-4N-acetylglucosaminyltransferase.

16 (Original). The method of claim 15, wherein the method further comprises contacting the product of the N-acetylglucosaminyltransferase reaction with a β 1-4mannosyltransferase and GDP-mannose to form a saccharide moiety that comprises Man β 1-4GlcNAc β 1-4GlcNAc-.

17 (Original). The method of claim 16, wherein the method further comprises contacting the Man β 1-4GlcNAc β 1-4GlcNAc- moiety with an α 1-3mannosyltransferase and GDP-mannose to form a saccharide moiety that comprises Man α 1-3Man β 1-4GlcNAc β 1-4GlcNAc-.

18 (Original). The method of claim 17, wherein the method further comprises contacting the Man α 1-3Man β 1-4GlcNAc β 1-4GlcNAc- moiety with an α 1-6mannosyltransferase and GDP-mannose to form a saccharide moiety that comprises Man α 1-6(Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc-.

19 (Original). The method of claim 18, wherein the method further comprises contacting the Man α 1-6(Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc- moiety with a β 1-2N-acetylglucosaminyltransferase and UDP-GlcNAc to form a saccharide moiety that comprises Man α 1-6(GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc-.

20 (Original). The method of claim 19, wherein the method further comprises contacting the Man α 1-6(GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc- moiety with a β 1-2N-acetylglucosaminyltransferase and UDP-GlcNAc to form a saccharide moiety that comprises GlcNAc β 1-2Man α 1-6(GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc-.

21 (Original). The method of claim 11, wherein the method further comprises contacting the saccharide moiety with one or more of a β 1-4N-acetylglucosaminyltransferase, an α 1,3fucosyltransferase, an α 1,2 fucosyltransferase, an α 1,4fucosyltransferase, a β 1-4galactosyltransferase, and a sialyltransferase, to form a biantennary or triantennary oligosaccharide structure.

22 (Currently Amended). The method of claim 1, wherein the [incorporating step is in vivo] translation system comprises a cell.

23 (Currently Amended). The method of claim 1, wherein the incorporating step comprises incorporating the unnatural amino acid into the protein with [using] an orthogonal tRNA/orthogonal aminoacyl-tRNA synthetase (O-tRNA/O-RS) pair, wherein the O-tRNA recognizes a selector codon and incorporates the unnatural amino acid into the protein in response to the selector codon, and wherein the O-RS [preferentially] aminoacylates the O-tRNA with the unnatural amino acid.

24 (Original). The method of claim 23, wherein the O-RS comprises an amino acid sequence comprising any one of SEQ ID NO.: 1, 2, or 3.

25 (Original). The method of claim 23, wherein the O-tRNA comprises a mutRNA^{Tyr}_{CUA} (SEQ ID NO. 7).

26-57 (Cancelled).

58 (New). The method of claim 1, wherein incorporating the unnatural amino acid into the protein comprises aminoacylating an OtRNA with an unnatural amino acid.

59 (New). The method of claim 1, wherein incorporating the unnatural amino acid into the protein comprises: aminoacylating an OtRNA with an unnatural amino acid, using an ORS, wherein the ORS aminoacylates the OtRNA more efficiently than the ORS aminoacylates any endogenous tRNA of the translation system and wherein the ORS aminoacylates the ORS more efficiently with the unnatural amino acid than with a natural amino acid.

60 (New). The method of claim 1 further comprising purifying the glycoprotein.